ABSTRACT

The invention relates to a protein which presents identical or different catalytically active domains of glycosyltransferases and has a processive action. In particular, the same protein is successively active in at least two successive process steps.

Tabl 1. ^{1}H-NMR data of peracetylated tetra- (<u>4</u>), tri- (<u>3</u>), and di-(<u>2</u>)glycosyldiacylglycerolipids isolated from *E. coli* BL21 (DE3) expressing *ypfP*. ß-Gentiobiose octaacetate (<u>1</u>) serves as reference substance. Spectra were recorded at 600 MHz and 300K; signals are referenced to internal TMS (0.000 *ppm*).

Sugar	Chemical shift of proton						
	1	2	3	4	5	6a	6b
	δ (*ppm*)						
Tetrasaccharide, <u>4</u>							
A ß-D-Glc-(1→	4.502	4.931	5.148	5.011	3.658	4.059	4.211
B →6)-ß-D-Glc-(1→	4.490	4.858	5.101	4.858	3.60*	3.56*	3.807
C →6)-ß-D-Glc-(1→	4.480	4.880	5.117	4.858	3.60*	3.56*	3.855
D →6)-ß-D-Glc-(1→Gro	4.412	4.845	5.117	4.875	3.60*	3.523	3.890
Trisaccharide, <u>3</u>							
A ß-D-Glc-(1→	4.506	4.916	5.140	5.006	3.646	4.065	4.202
B →6)-ß-D-Glc-(1→	4.462	4.866	5.095	4.857	3.60*	3.55*	3.811
C →6)-ß-D-Glc-(1→Gro	4.417	4.871	5.117	4.848	3.59*	3.512	3.878
Disaccharide, <u>2</u>							
A ß-D-Glc-(1→	4.508	4.913	5.121	5.006	3.639	4.056	4.210
B →6)-ß-D-Glc-(1→Gro	4.409	4.862	5.112	4.828	3.60*	3.538	3.802
ß-Gentiobiose octaacetate, <u>1</u>							
A ß-D-Glc-(1→	4.465	4.902	5.107	4.976	3.586	4.038	4.176
B →6)-ß-D-Glc-(1→	5.608	5.001	5.147	4.911	3.708	3.496	3.845

Sugar	Coupling constant *J* (Hz)						
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{6a,6b}$	$J_{6b,5}$
Tetrasaccharide, <u>4</u>							
A ß-D-Glc-(1→	7.8	9.5	9.6	9.7	2.0	12.2	5.2
B →6)-ß-D-Glc-(1→	7.6	9.7	9.9	10.0	-	10.4	2.3
C →6)-ß-D-Glc-(1→	7.6	10.0	9.9	10.0	-	10.9	2.5
D →6)-ß-D-Glc-(1→Gro	8.0	9.8	9.9	9.8	4.6	10.4	2.1
Trisaccharide, <u>3</u>							
A ß-D-Glc-(1→	7.9	9.5	9.6	9.7	2.4	12.1	4.9
B →6)-ß-D-Glc-(1→	8.0	9.7	9.4	9.8	6.9	-	2.2
C →6)-ß-D-Glc-(1→Gro	8.0	10.0	9.5	9.7	-	11.5	2.6
Disaccharide, <u>2</u>							
A ß-D-Glc-(1→	8.0	9.3	9.6	9.7	2.1	12.3	4.8
B →6)-ß-D-Glc-(1→Gro	7.9	9.9	9.5	10.0	6.8	10.8	2.0
ß-Gentiobiose octaacetate, <u>1</u>							
A ß-D-Glc-(1→	8.0	9.6	9.5	9.6	2.4	12.3	4.8
B →6)-ß-D-Glc-(1→	8.2	9.6	9.5	9.7	2.4	11.4	5.7

Other signals: <u>4</u>, OAc 2.030,1.993 (2x),1.976 (2x), 1.969, 1.963, 1.959, 1.937, 1.932,1.917 (2x); $-CH_2-$ 1.185, CH_3 ; -CH=CH- 5.277. <u>3</u>, OAc 2.208, 1.991, 1.974 (2x), 1.967, 1.955 (2x), 1.930, 1.927, 1.914, $-CH_2-$ 1.185, $-CH_3$ 0.812, -CH=CH- 5.277. <u>2</u>, OAc 2.025, 1.973 (2x), 1.958, 1.953, 1.928, 1.924, $-CH_2-$ 1.185, $-CH_3$ 0.812, -CH=CH- 5.278. <u>1</u> OAc 2.028, 2.010, 1.992, 1.954, 1.943, 1.936, 1.920 (2x) ppm.

* non resolved multiplets

Tabl 2. 600-MHz ^{1}H-NMR data of **PL1**$_{Ac,Me}$ and **PL2**$_{Ac,Me}$ and their diastereomers **PL1'**$_{Ac,Me}$ and **PL2'**$_{Ac,Me}$ ($CDCl_3$, 300K; internal TMS, δ_H = 0.000).

	PL2		**PL2'**		**PL1**		**PL1'**	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
→6)-ß-D-Glc^B-(1→								
H-1	4.407	$J_{1,2}$ 8.0	4.416	$J_{1',2'}$ 8.0				
H-2	4.856	$J_{2,3}$ 9.2	4.853	$J_{2',3}$ 9.2				
H-3	5.110	$J_{3,4}$ 9.6	5.113	$J_{3',4'}$ 9.6				
H-4	4.828	$J_{4,5}$ 9.5	4.816	$J_{4',5'}$ 9.5				
H-5	3.585	$J_{5,6a}$ 6.8	3.598	$J_{5',6a}$ 6.3				
H-6a	3.530	$J_{6a,6b}$ 11.2	3.519	$J_{6'a,6'b}$ 10.8				
H-6b	3.826	$J_{5,6b}$ 2.5	3.839	$J_{5',6'b}$ 2.5				
ß-D-Glc^A-(1→								
H-1	4.531	$J_{1,2}$ 8.0	4.513	$J_{1',2'}$ 8.0	4.461	$J_{1,2}$ 7.9	4.4457	$J_{1',2'}$ 7.9
H-2	4.900	$J_{2,3}$ 9.4	4.989	$J_{2',3'}$ 9.4	4.904	$J_{2,3}$ 9.5		
H-3	5.125	$J_{3,4}$ 9.4	5.125	$J_{3',4'}$ 9.4	5.131	$J_{3,4}$ 9.6		
H-4	4.992	$J_{4,5}$ 9.8	4.973	$J_{4',5'}$ 9.8	5.002	$J_{4,5}$ 9.7		
H-5	3.655		3.647		3.643	$J_{5,6a}$ 2.6		
H-6a	4.01*		4.01*		4.068	$J_{6a,6b}$ 12.4		
H-6b	4.10*		4.10*		4.183	$J_{5,6b}$ 4.9		
P→3)-Gro^1								
H-1a	4.10*		4.10*		4.11*	$J_{1a,1b}$ 11.9	4.11*	$J_{1'a,1b'}$ 12.4
						$J_{1a,2}$ 4.8		
H-1b	4.261	$J_{1b,2}$ 3.9	4.264	$J_{1'b,2'}$ 3.9	4.264	$J_{1b,2}$ 4.6	4.273	$J_{1'b,2'}$ 4.6
H-2	5.221		5.221		5.164	$J_{2,3a}$ 4.9	5.164	
						$J_{2,3b}$ 5.3		
H-3a	4.07*	$J_{3a,3b}$ 12.0	4.07*	$J_{3'a,3'b}$12.1.	4.09*		4.09*	
H-3b	4.14*		4.14*		4.12*		4.11*	
P→1)-Gro^2								
H-1a	4.07*	$J_{1a,2}$ 12.2	4.07*		4.07*		4.06*	
H-1b	4.223	$J_{1b,2}$ 3.5	4.228	$J_{1'b,2'}$ 3.6	4.11*		4.11*	
H-2	5.11*		5.11*		5.082	$J_{2,3a}$ 4.9	5.082	$J_{2',3'a}$ 5.3
H-3°	3.554	$J_{3a,3b}$ 10.8	3.559	$J_{3'a,3'b}$10.4	3.668	$J_{3a,3b}$ 11.0	3.697	$J_{3'a,3b'}$ 11.0
H-3b	3.826	$J_{3b,2}$ 4.8	3.901	$J_{3',2'}$ 4.6	3.868		3.872	
Methyl phosphate								
P-OCH_3	3.720	$^3J_{P,H}$ 9.3	3.701	$^3J_{P,H}$ 9.2	3.711	$^3J_{P,H}$ 11.1	3.692	$^3J_{P,H}$ 11.2
Fatty acids								
-CH=CH-	5.277				5.28*			
$-CH_2$-CH=	1.943				1.940			
$-CH_2$- (α)	2.230, 2.258				2.242, 2.266			
$-CH_2$- (ß)	1.538				1.536, 1.548			
$-CH_2$- (γ/ω−1)	1.22 – 1.26				1.18 – 1.27			
$-CH_3$ (ω)	0.811, 0.815	J 6.3			0.810, 0.824	J 6.3		

Additional signals for OAc: 2.025, 2.018, 1.989, 1.954, 1.934 ppm in **PL1** and **PL1'**; 1.975 (2x), 1.972, 1.965, 1.927, 1.921 ppm in **PL2** and **PL2'**; * non resolved multiplet.

Tabl 3. 90.6-MHz ^{13}C-NMR data of **$PL1_{Ac,Me}$**, **$PL2_{Ac,Me}$**, $MGlcD_{Ac}$, and $DGlcD_{Ac}$ ($CDCl_3$, 300K; internal $CDCl_3$, δ_H = 77.0).

	PL1		PL2[§]	MGlcD[§]	DGlcD
	δ (ppm)	*J* (Hz)	δ (ppm)	δ (ppm)	δ (ppm)
→6)-ß-D-Glc^{B}-(1→					
C-1			101.1		100.7
C-2			71.1		71.1
C-3			72.6		72.6
C-4			69.0		69.1
C-5			73.4		73.3
C-6			68.0		68.1
ß-D-Glc^{A}-(1→					
C-1	101.1		101.2	101.3	100.8
C-2	71.1		71.1	71.2	71.1
C-3	72.6		72.6	72.7	72.7
C-4	68.3		68.4	68.5	68.3
C-5	72.0		72.2	72.1	72.0
C-6	61.8		65.7	61.8	61.8
P→3)-Gro^{1}					
C-1	61.7		61.6		
C-2	69.4	$^{3}J_{C,P}$ 6	69.3		
C-3	65.6	$^{4}J_{C,P}$ ~6	65.8		
P→1)-Gro^{2}					
C-1	65.7	$^{4}J_{C,P}$ ~ 6	62.2	62.1	62.3
C-2	70.3	$^{3}J_{C,P}$ 7.6	69.5	69.7	69.6
C-3	67.1		67.5	67.8	67.5
P-OCH_3	54,6, 54.5				
Fatty acids					
-CH=CH-	128.1		n.d.	n.d.	130.0
	127.8		n.d.	n.d.	129.9
	127.5		n.d.	n.d.	129.8
-CH_2-CH=	27.2		27.2	27.4	27.2
C-1	173.2, 173.8		n.d.	n.d.	173.3, 173.8
C-2	34.0, 34.1		34.0	34.4	34.2, 34.6
C-3	24.8		24.9	25.0	24.9
C-4..C-13/16	29.7... 28.9		29.9...29.1	30.4...29.0	29.9...29.1
C-(ω3)	31.8, 31.9		31.9	31.3	31.9
C-(ω2)	22.7, 22.6		22.7	19.8	20.6, 20.7
C-(ω1)	14.1		14.1	14.2	13.1

Additional signals for OAc in **$PL1_{Ac,Me}$**: $COCH_3$ at 170.6, 170.2, 169.9, 169.4, 169.2 ppm; $COCH_3$ at 20.8, 20.7, 20.6 (5x) ppm. [§] Values taken from HMQC experiments; n.d., not determined.